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SOME CHARACTERISTICS OF THE STREPTOCOCCI FOUND IN SCARLET FEVER.*†

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THE following work was undertaken to determine the frequency of the occurrence of the hemolysing streptococcus in the blood, pus, blister fluid, and throats of scarlet fever patients, its relative proportion to other streptococci present, and the persistence of its hemolysing power. By the term "hemolysing streptococcus" is meant that spherical or spheroidal organism which, by dividing in only one plane, grows in long or short chains, the individuals usually occurring in pairs, and which gives a more or less definite area of hemolysis about each colony when plated out on blood agar.

In 1903 Schottmüller,[1] and soon after, Rosenow,[2] showed that pneumococcus colonies gave green color in blood agar poured plates, *Strept. pyogenes* gave hemolysing colonies, and other streptococci (*Strept. viridans*), green colonies. Ruediger,[3] working with various throat cultures, found that pneumococci showed green colonies and sometimes hemolysing colonies; *Strept. pyogenes*, hemolysing colonies; and other streptococci, green colonies. He also found that hemolysing colonies may show green color in subsequent plates, and that slightly hemolysing colonies and those with greenish cast, while very puzzling, should be distinguished from *Strept. pyogenes*. Moreover, he found but a small proportion of the green colonies occurring in scarlet fever and other throat cultures to be pneumococcus. The others seemed to belong to a large group lying between *Strept. pyogenes* and the pneumococcus, some being very like *Strept. viridans* of Schottmüller and others being related to pneumococci.

Ruediger explains the green coloration in blood plates as due to the formation of lactic acid, which acts then on the sugars in the blood medium. In glucose-agar blood plates *Strept. pyogenes* does give green colonies entirely, but none in blood and plain agar because it

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Münch. med. Wchnschr., 1903, 50, pp. 849, 909.

[2] *Jour. Infect. Dis.*, 1904, 1, p. 308.

[3] *Ibid.*, 1906, 3, p. 663.

does not ferment muscle sugar readily and the hemolysis outruns the green coloration. In our laboratory all the hemolysing strains, and also the green ones, when tested and retested in glucose-agar blood plates, gave green colonies.

This plate method was the chief means of isolating the streptococci in pure culture from the scarlet fever material. In the case of blood about 5 or 6 c.c. were drawn from the veins of the arms of living patients, and in autopsy cases from the heart by means of a sterile syringe.

The blister fluid was obtained by applying strong ammonia to a small area of the skin where the rash was brightest, usually on the chest, by means of a disk of blotting paper surrounded by a circle of vaseline and held firmly in place by crossed adhesive strips arranged in a star form, so that the air was practically excluded. After 10 or 15 minutes the fluid in the blister thus formed was drawn with sterile capillary pipettes which penetrated the delicate epidermis easily. Cultures were made in serum broth, and, after 24 hours' incubation, were plated out in plates made with horses' blood.

The pus was drawn by a sterile syringe from suppurating glands or other foci of infection. Dilutions were made, but were plated out at once because the number of organisms present was usually much greater than in the blood.

Throat cultures were taken by means of sterile cotton swabs rubbed thoroughly on both tonsils. The swab of each case was beaten in a tube of plain broth and dilutions made before plating.

The following table gives in condensed form the number of cases examined and strains isolated in pure culture, from material taken from early scarlet fever and measles cases.

TABLE 1.

STREPTOCOCCI IN SCARLET FEVER.

SOURCE	NUMBER OF CASES TESTED	NO GROWTH	STREPTOCOCCI ISOLATED	BLOOD PLATES	
				Strept. pyogenes? Hemolysing	Other Streptococci
Autopsy blood (from heart)	18	8	10	+	–
Living blood (from arm)	16	15	1	–	+
Total	34	..	11		
Autopsy pus	10	3	7	+	–
Living pus	9	0	9	+	+ 1 colony in 1 case
Total	19	..	16		
Blister fluid	26	23	3	+	+
Scarlet throats	35 (5 rejected)	0	30	+ (22 cases)	+ (26 cases)
CONTROLS:					
Measles throats	24	..	24	+ (20 cases)	+ (24 cases)
Diphtheria throats	4	..	4	+ (a few colonies in one case)	+ (4 cases)

The percentage of hemolysing colonies in the different strains of both scarlet and measles throats varied markedly, namely, from zero to one hundred. Of the 30 scarlet fever throats, 8 cases showed no hemolysing colonies at all; 4 showed no green colonies, and 18 showed both green and hemolysing colonies. Of the 24 measles throats, all showed green colonies and 4 showed no hemolysing colonies. There was so much variation in color reaction and the area of hemolysis, that the following terms were used in an attempt to classify the colonies:

Hemolysing, small hemolysing, very small hemolysing.
Slightly hemolysing (large and small).
Doubtful.
Green (light and dark).

Colonies were fished and replated during successive generations and it was found that they varied up and down the scale.

Hemolysing colonies varied in area of hemolysis.

Green colonies varied in shade of green.

Small hemolysing colonies became large or slightly hemolysing, or green; this green in subsequent generations became hemolysing again.

Slightly hemolysing colonies remained the same, became doubtful, or showed green in later plates.

Doubtful colonies became slightly hemolysing, remained doubtful, or showed green.

The 30 streptococcus strains isolated from blood, pus, and blister fluid of scarlet fever cases were studied with two controls: C. 100, a Bellevue Laboratory culture, and C. 101, from a case of cellulitis after erysipelas. In contrast to the throat cultures, these strains from blood and pus, when tested in pure culture in blood plates, each gave good hemolysing colonies with one exception.[1] The exception was the only strain isolated from living blood, and it gave green colonies alone. The throat culture in this case also showed green colonies only. The patient from whom these cultures were derived had a good rash, ran a typical course of scarlet fever, and recovered without complications.

The three blister fluids which yielded streptococci showed both hemolysing and green colonies. These children recovered also.

In eight of the autopsy cases where both blood and pus were taken from the one subject, both materials were positive in four. In the other four the blood was negative in three with the pus positive in two. One case had negative pus and positive blood; another showed both the blood and pus negative. Blister fluid was obtained in only two cases from which blood and pus were taken later at autopsy. In both these cases the blister fluid was negative.

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[1] In addition, the pus from the cervical gland in one living case, Sc. 95, showed one green colony which remained green when retested twice.

RETEST OF THE HEMOLYSING POWER OF STREPTOCOCCUS STRAINS.

Twenty-five of these strains from scarlet fever cases and the two controls, C. 100 and C. 101, were kept in the ice-box on blood agar slants and transferred at about four-week intervals, for three months to a year. They were then retested in successive blood plates and showed marked variation as to hemolysis though all, except 51 Blood and Throat (which were green when isolated and remained so), had been fished from colonies which showed good hemolysis in the original. The retest was carried on as follows:

Technic.—From a 24-hour blood agar slant of each strain a very small portion of the growth was removed with a straight platinum needle and put in a 5 c.c. broth tube. This was shaken thoroughly and the two loopfuls of the broth transferred at once to a tube containing 1 c.c. of sterile horse blood. Melted agar was added and the plates poured. These plates were incubated 18 to 24 hours and the colonies showing least amount of hemolysis were fished on blood agar slants which were plated out in turn. A careful record was kept as to the size and reaction of the colonies of each strain, only the most variable fishings being carried on from one plating to another. Each broth tube, from which an agar plate was made, was incubated and a smear from it examined, so no contamination could creep in unawares.

From the hemolysing colonies arose:

1. Hemolysing colonies of similar size.
2. Slightly hemolysing (large and small).
3. Doubtful colonies.
4. Green (light and dark).

In subsequent platings, as in the case of fishings from the original throat cultures, there was a marked variation up and down the scale. Hemolysing colonies varied in hemolysis, became slightly hemolysing, doubtful, green, or showed no green nor hemolysis. Slightly hemolysing colonies became hemolysing, remained slightly hemolysing, or were doubtful, green, or showed no green nor hemolysis.

Green colonies remained green for the most part, but a few returned to hemolysing, slightly hemolysing colonies, or were doubtful.

In two strains, 53 Blood and Sc. 48 Blood, at the fourth and third platings, respectively, there occurred among other colonies some which showed neither hemolysis nor green color. These were carefully fished and found in smears of broth transfers to be pure streptococcus. When these fishings were replated and refished one or more times they gave rise to hemolysing, slightly hemolysing, green, and doubtful, sometimes all in the same plate. Only a few remained the same, that is, showed no green nor hemolysis.

Eight fishings from these colonies (of 53 Blood), which showed no green color and no hemolysis, were tested simultaneously in blood plates of plain agar, peptone water agar, and sugar-free agar (made from broth with colon bacillus added and resterilized), with the result that in the plain and peptone agar the hemolysis returned for the most part, while in the sugar-free agar the colonies remained the same or showed but slight trace of hemolysis.

These colonies (showing no green nor hemolysis) were tested also upon: Glucose agar which gave green colonies; glycerin agar which gave hemolysing and slightly hemolysing colonies; beerwort agar which gave deep-green colonies.

Two strains, 13 Blood and Pus, were kept on glucose-agar blood slants for a number of transfers at several-day intervals and when changed suddenly to plain agar blood plates showed no loss of hemolysing power.

The two control cultures, one (C. 100) from the laboratory of Bellevue Hospital, and the other (C. 101) isolated recently from a case of cellulitis following erysipelas, showed but little variation, for only two slightly hemolysing colonies occurred in the six platings of C. 100 and none in C. 101.

A retest was made also of 20 fishings from scarlet fever throat cultures. Control Sc. 40 was from throat of an erysipelas case which developed a rash and was suspected of being scarlet fever, but later proved negative. These strains had been kept in the ice-box on blood agar slants for 12 to 18 months, and comprised not only hemolysing, but hemolysing and green colonies when first isolated. The reactions of the originally hemolysing colonies followed practically those showed of the blood and pus hemolysing colonies when they were retested. The colonies which had been green when isolated remained so in every replating except once in the case Sc. 87, where doubtful colonies occurred in the third plate, but when these were fished and plated again they showed green and remained so for subsequent platings.

In the replating of the two slightly hemolysing strains, Sc. 81, throat, and Sc. 88, throat, greater variation occurred. *Sc. 88 slightly hemolysing* (large) gave: Hemolysing, slightly hemolysing, and a few doubtful colonies, which, however, became hemolysing again. On the other hand, *Sc. 81 slightly hemolysing* gave but a low percentage of hemolysing colonies and these were small in size. The majority of colonies were slightly hemolysing with a few doubtful and a number of green ones. These last, the green, became small and very small hemolysing colonies four times, but for the most part gave rise to slightly hemolysing and still other green colonies.

The control from the erysipelas throat, which was hemolysing when isolated, gave, in the retest, large hemolysing colonies.

SUMMARY.

Although the power of hemolysing streptococci to cause hemolysis seems to be a slightly variable quality and is certainly dependent upon conditions we do not understand, yet there is a distinct tendency in this power to continue, even though the strains be kept on artificial media, for in the retest the majority of colonies were hemolysing, the variation from the original strain being usually about 5 per cent, or less, in the first replating. In these and subsequent plates only the most variable colonies were fished and even then the percentage of hemolysing colonies was usually greater than 25 per cent, sometimes running between 75 and 100 per cent. All strains were plated out at least six successive times and some, eight or nine times. Any variation occurring cannot be due entirely to long cultivation on artificial media, partly because some small hemolysing colonies from throat cultures changed rapidly to green after isolation; and also some recent green colonies, derived from old hemolysing cultures kept on agar for seven to twelve months, have regained their hemolysis once more. To be sure, when some of these apparently accidental green colonies, which formed but a very small part of all the colonies present, were selected strains were brought out which had lost their hemolysis completely. This occurred in 33½ per cent of the 25 Blood and Pus cultures retested, and these green strains failed to regain any appearance of hemolysis, though they were plated out six successive times. It is doubtful if these can longer be classed with *Strept. pyogenes.* On the other hand, as cited above in Sc. 48 Blood, some green colonies show a quick change to hemolysis again.

The occurrence of hemolysing streptococci (*Strept. pyogenes?*), as seen in Table 1, was almost constant in the material taken from scarlet fever, measles, and diphtheria throats, while *Strept. viridans* was always present.

In scarlet fever cases the hemolysing streptococcus alone[1] occurred in the pus from cervical glands and other suppurating foci in all of the nine living cases, and was recovered in 7 of the 10 autopsies.

In the living blood and blister fluid streptococci would seem to be rather infrequent since they were found in but 3 out of 26 blisters and once in 16 samples of living blood. It is interesting to note that the three children from whom streptococci, both hemolysing and viridans, were isolated by blistering and also the man whose blood contained *Strept. viridans*, all recovered.

Streptococci were not found in all cases in which blood was examined after death, being present in but 10 of the 18 cases tested. These were of the hemolysing type.

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[1] One case had one green colony, which remained green when retested.